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How do light harvesting proteins support long-lived quantum coherences?

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14. ABSTRACT
Cryptophyte algae use light harvesting proteins to facilitate photosynthesis under low light conditions. Two-dimensional electronic spectroscopy (2DES) of these proteins indicates that they utilize non-trivial quantum coherence phenomena in their function. Our aim is to probe the structural origin of these quantum effects. We have previously discovered that these algae can produce two structurally distinct light harvesting proteins: a closed form that supports quantum coherence and an open form that does not. To determine the structural basis for these two forms, our aim is to generate hybrid proteins via synthetic biology approaches. We have shown that we can fully unfold and separate the alpha and beta subunits that make up the light harvesting proteins. We can then recombine these and refold the proteins. The refolded wild type proteins show the same 2DES are the natural proteins. We have then created hybrid proteins taking either alpha or beta subunits from open and closed forms and recombining them with the complementary subunit from the opposite form. These hybrid proteins refold as determined by both optical and far UV circular dichroism spectroscopy. The hybrid proteins differ from wild type proteins in that they elute from size exclusion chromatography as if they are heterodimers rather than the wild type heterotetramers. We have succeeded in crystallizing hybrid proteins for high resolution structure determination. The hybrid proteins are soluble and stable and should be amenable to 2DES for evaluation of quantum coherences.

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Abstract:

Cryptophyte algae use light harvesting proteins to facilitate photosynthesis under low light conditions. Two-dimensional electronic spectroscopy (2DES) of these proteins indicates that they utilize non-trivial quantum coherence phenomena in their function. Our aim is to probe the structural origin of these quantum effects. We have previously discovered that these algae can produce two structurally distinct light harvesting proteins: a closed form that supports quantum coherence and an open form that does not. To determine the structural basis for these two forms, our aim is to generate hybrid proteins via synthetic biology approaches. We have shown that we can fully unfold and separate the alpha and beta subunits that make up the light harvesting proteins. We can then recombine these and refold the proteins. The refolded wild type proteins show the same 2DES are the natural proteins. We have then created hybrid proteins taking either alpha or beta subunits from open and closed forms and recombining them with the complementary subunit from the opposite form. These hybrid proteins refold as determined by both optical and far UV circular dichroism spectroscopy. The hybrid proteins differ from wild type proteins in that they elute from size exclusion chromatography as if they are heterodimers rather than the wild type heterotetramers. We have succeeded in crystallizing hybrid proteins for high resolution structure determination. The hybrid proteins are soluble and stable and should be amenable to 2DES for evaluation of quantum coherences.

Introduction:

Photosynthesis is central to life as we know it. It is the key source of energy to our biosphere. The engine of photosynthesis is a protein complex known as the reaction centre (RC). In plants, photosynthetic bacteria and algae, the RC converts light energy into a charge separation that then powers biochemical pathways to lock energy in chemical form so as to support all other biological processes. However, sunlight does not directly stimulate RC, but it is harvested by complexes of antenna proteins that capture light energy and transfer it to RC. The importance of these light harvesting proteins is that they achieve a very efficient means to trap sunlight, with a photon efficiency approaching one, i.e. every photon that reaches the system is trapped. How do biological light harvesting systems achieve near perfect quantum efficiency?

Cryptophyte algae are playing a central role in unravelling the mechanism by which this

quantum efficiency is achieved. A central question is: does quantum mechanics play a non-trivial role in biological processes such as light harvesting[1]? In 2010, we published the first observation under near physiological conditions of quantum beats in the 2D electronic spectra of a protein system, the cryptophyte light harvesting protein[2]. This landmark paper raised the possibility that quantum mechanical processes may be at the heart of light harvesting, possibly responsible for the near perfect quantum efficiency of these systems[1,3,4].

Two central questions arise from the observation of quantum phenomena in the cryptophyte light harvesting proteins: (1) what is the structural basis for maintaining long-lived quantum states in the proteins? and (2) are these quantum mechanical phenomena under biological control? Answering these questions will move us closer to understanding the biological significance of quantum effects for light harvesting efficiency. They will also provide the first indications of how one could engineer such systems for technological applications.

Cryptophytes are single-celled algae that have evolved photosynthetic systems from red algae via endosymbiosis, which in turn, have evolved from cyanobacteria via endosymbiosis[5]. All three use light harvesting systems based on globin-fold proteins known as phycobiliproteins (PBPs)[6,7]. These proteins contain one to three covalently-bound tetrapyrrole chromophores that are responsible for light harvesting. In red algae and cyanobacteria, the PBPs are arranged into hexameric rings that are stacked into rod-like antenna complexes (phycobilisomes) attached to the outside of the thylakoid membrane[7,8].

Evolution of cryptophytes is concomitant with a radical rearrangement of the parent red algal light harvesting system. Instead of antenna rods, the PBPs form soluble, dimeric proteins that fill the lumen of the thylakoid[9,10]. To form dimers, the globin-fold β subunits are non-covalently linked to extended α subunits to form an αβ monomer. The α subunits are short (~70 residues) with a single chromophore covalently attached near the N-terminus. We determined the first crystal structure of the resulting $\alpha_1\beta.\alpha_2\beta$ heterodimer[11,12]. This structure demonstrated that the cryptophyte PBP had evolved by the rearrangement of subunits. One striking feature of the structure was that two chromophores are in physical contact on the pseudo two-fold axis, a feature that had never been seen before and was likely to be important in electronic coupling and light harvesting.

The cryptophyte light harvesting proteins offer a unique opportunity to dissect the relationship between protein structure and quantum effects. The cryptophyte system has evolved from the light harvesting antenna complex (phycobilisome) of cyanobacteria and red algae. It has maintained the basic structural unit (a globin fold protein with three chromophores) but rearranged its quaternary structure. In addition, we have recently discovered that there are two distinct arrangements of this protein in different cryptophytes[13]. The difference between these two structures appears to be due to the insertion of a single amino acid in the small, alpha subunit of the protein[13]. Thus, evolution has provided a set of protein-chromophore arrangements that all function as light harvesting antennae. The key is to understand how these arrangements alter energy transfer, in particular quantum effects, and how this affects the mechanism and efficiency of energy transfer and light harvesting.

The aim of this project will be to initiate a synthetic biology approach to answering the question: what is the structural basis for the long-lived coherent states that are observed in the

multi-dimensional femtosecond laser spectroscopy of cryptophyte light harvesting proteins? Our data based on the crystal structures of several related light harvesting proteins indicates that the insertion of a single amino acid in the small α subunit of the protein results in a dramatic structural change comprising a ~70° rotation between the two halves of the protein[13]. Preliminary two-dimensional electronic spectroscopy indicates that this structural change switches the protein between a form that sustains long-lived quantum coherence to a form that does not[13]. Our current goal is to prove that this structural transition does indeed preclude quantum coherence. If this is the case, then we should be able to determine the precise structural factors that support long-lived coherence.

At the core of this project, we have recently discovered that one of the cryptophyte light harvesting proteins can be completely unfolded and then refolded into a fully functional form. We will use this as a platform to generate synthetic chimera proteins by substituting the α subunits from the wild type with other α subunits. Thus, we should be able to create pairs of light harvesting proteins that differ only in the sequence of the small α subunits. By choosing pairs of α subunits that differ in the presence or absence of the critical aspartate residue (the one that results in the ~70° rotation between αβ monomers), we should be able to generate matched protein pairs that differ mainly in their structure.

Experiment:

Formation of Hybrid Proteins

To create synthetic hybrid phycobiliproteins, subunits of different open and closed form phycobiliproteins were refolded after the subunits are purified by HPLC. Once the subunits are mixed, they are subsequently dialysed into 4:1 (v/v) CH_3CN in 0.1 M $HCl_{(aq)}$ at pH 2, and refolding requires an additional 20 fold dilution out the organic solvent to reform the native proteins.

Results and Discussion:

Reversible refolding of cryptophyte light harvesting proteins
(taken from the submitted paper:
Laos, A.J., Dean, J. C., Toa, Z.S.D., Wilk, K.E., Scholes, G.D., Curmi, P.M.G. & Thordarson, P. "Cooperative subunit refolding of a light-harvesting protein through a self-chaperone mechanism". Under review.)

Using pH titrations from pH 7 to pH 2, and then back to pH 7, we have shown the ability of the light-harvesting PE545 phycobiliprotein to reassemble and refold from unfolded polypeptide states of the α and β-subunits without chaperones (Figure 1 – left side pathway: B→C→D→G→I). Denaturation and renaturation of PE545 was performed in 25 mM potassium phosphate buffer via simple titrations with hydrochloric acid to pH 2 and a subsequent titration back to pH 7 with sodium hydroxide after a time period of 10 minutes resulting in ca 80% yield for the recombined, refolded protein (Figure 1G). Leaving the proteins for longer than 10 min. at low pH reduced the yield of the refolded proteins while other refolding methods such as cooling

after heat denaturation proved irreversible. At acidic pH, the β-subunits remain relatively water soluble and stable for short time periods (< 60 min), while the water soluble α-subunits remain stable throughout. Both subunits are positively charged at pH 2 (β subunit: +12; α_1: +7.5; and α_2: +8, including the tetrapyrrole chromophores).

To characterize these unfolded states we performed a wide range of steady state spectroscopy on the native, unfolded, and refolded proteins, (Figure 1G) along with their purified subunits from reverse phase HPLC using 1:2:12 (v/v) CH_3CN/isopropanol/0.1 M $HCl_{(aq)}$ as the running buffer (Figure 1F). Their purified α/β-subunits allowed deconvolution of the relative spectral contributions of the subunits in all states from the unfolded to native state, which further aided in elucidating the refolding events. Further, refolding experiments of the subunits show that the purified, isolated β-subunits refold (see below) whereas the α-subunits do not. When the two reverse phase HPLC separated subunits α and β were isolated at pH 2 and recombined at pH 7 (Figure 1, right side pathway: B→C→F→H→I) they refolded correctly to the native PE545 structure (Figure 1H). We performed the same pH titrations and reverse phase HPLC separation experiments on the open form PC577 phycobiliprotein as described above for the PE545 protein. Whether PC577 was titrated from pH 7 to pH 2, with or without reverse phase HPLC separation of subunits, and then titrated back to pH 7, the protein recovered and appeared spectroscopically identical to native PC577.

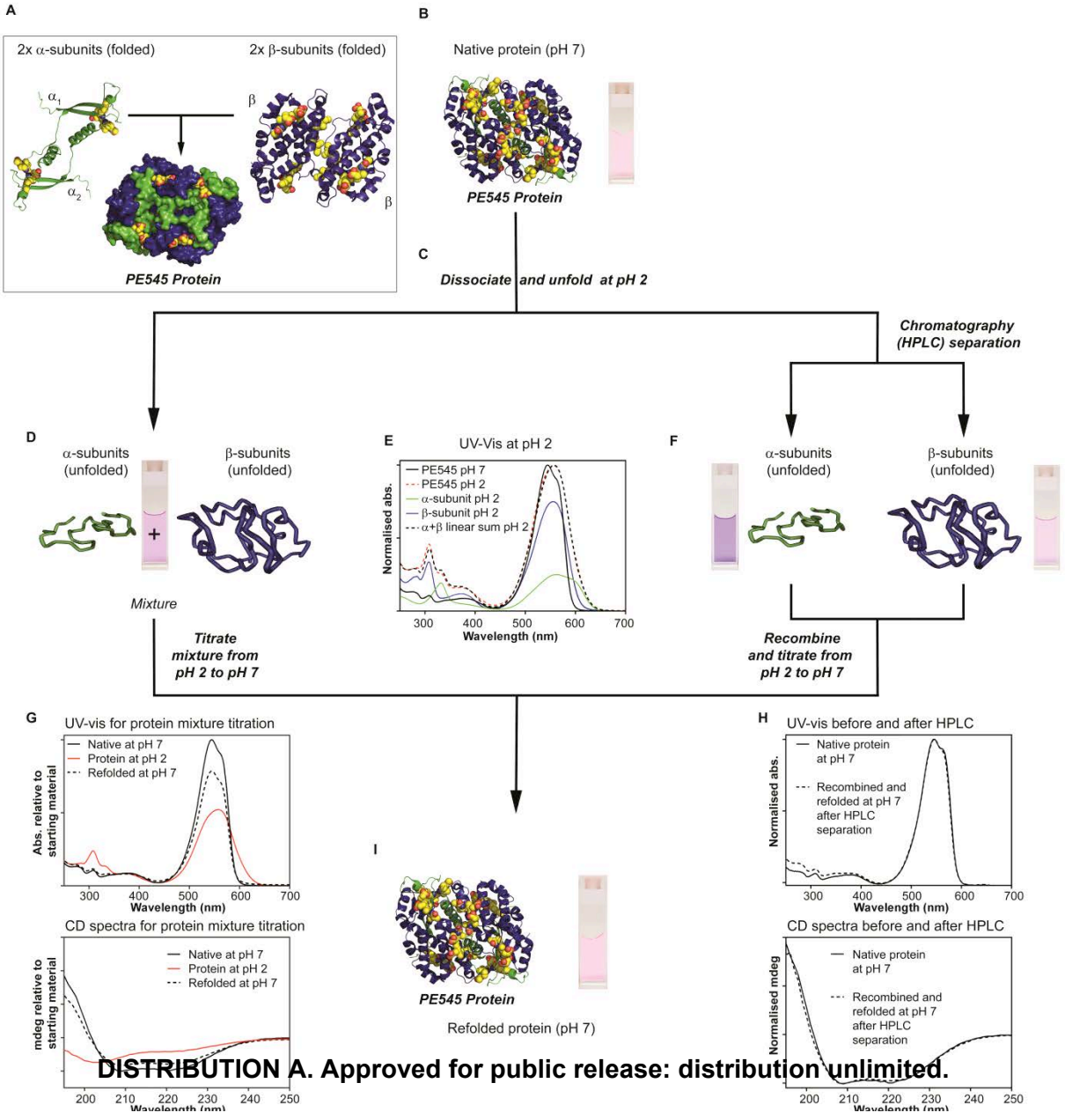

Figure 1. *Unfolding and refolding of light-harvesting phycoerythrin 545 (PE545). A) The α- and β-subunits from the crystal structure of PE545 and the corresponding solvent accessible surface of the whole protein (pdb: 1XF6). C) Lowering the pH to 2 leads to dissociation and unfolding of the PE545 protein. D) The solution of the PE545 after unfolding at pH 2. E) The normalized UV-Vis spectra at pH 2 of the unfolded protein in aqueous solution (red dotted line) represented schematically in D), as well as F, the reverse phase HPLC separated α-subunits (green line) and the β-subunits (blue line) from F in 4:1 (v/v) CH_3CN in 0.1 M $HCl_{(aq)}$, and the linear sum (dotted black line) of the α- and β-subunits spectra from F. G) The UV-Vis (upper panel) and CD (lower panel) spectra of the native protein (black line) from B, the unfolded protein (red line) from D, and refolded protein mixture at pH 7 (dotted black line). H) The UV-Vis (upper panel) and CD spectra of the native protein (solid line) from A, and recombined pure α- and β-subunits from F, after titrating it back to pH 7 (dotted line). I) A solution of the refolded protein from H, at pH 7.*

To provide an internal characterization of the refolded states of the light-harvesting phycobiliproteins we compared coherent oscillations recorded in the time-domain (and their frequency content in the spectral domain) for the native and refolded proteins using coherent electronic spectroscopy. The coherences activated by the broadband pulse(s) serve as fingerprints of each chromophore within the protein assembly as their frequencies and amplitudes are inherently sensitive to the chromophore conformation and its local protein environment. In this way, coherent electronic spectroscopy can be utilized for its inherent selectivity of the eight structurally-distributed tetrapyrroles, exploiting them as local structural probes of the protein scaffold itself (Figure 2). Specifically, we implemented broadband transient absorption (BBTA) spectroscopy, where the coherent oscillations along the pump-probe time delay are recorded as a function of probe frequency (ν_3) throughout the UV-Vis absorption spectrum, and two-dimensional electronic spectroscopy (2DES), which further disperses the BBTA signal into two dimensions by resolving the excitation axis (ν_1) in addition to the detection axis (ν_3). The combination of both methods allowed us to decisively compare coherences for the proteins from ultrafast (fs) to picosecond timescales.

As an example, the total 2D (magnitude) spectra at pump-probe time delay t_2 = 100 fs (Figure 2A), and 2D oscillation maps showing the region in the 2D spectrum where oscillatory amplitude is prominent at 500 cm^{-1} (Figure 2B), are compared. The data recorded for native and refolded PE545 protein are nearly identical. More importantly, the 1D traces (Figure 2C) of the BBTA spectra and their Fourier (FT) power spectra (Figure 2D) show that the fundamental coherences in the native and refolded are almost identical out to 2 ps. This is notable considering that coherences in and between protein chromophores are highly dependent on their conformational/torsional states. The measurements show that the proteins refold with high fidelity. This is demonstrated by comparing the PE545 power spectrum to that of the α-subunit. Similar broadband femtosecond coherent spectroscopy experiments on the open form PC577 protein also show excellent agreement between the native and refolded protein.

The high spectral yield (Figure 1G) and equivalence among coherences of the refolded and native open/closed proteins (Figure 2) indicates that refolding and subunit assembly proceeds via a well-defined thermodynamic and kinetic funnel. We propose that this route is driven by autonomous refolding of the β-subunit. Evidence of this can be seen in the CD spectra. At pH 7, the β-subunit secondary structure is reformed, and more importantly, the visible CD band regains strength as the β-subunit backbone refolds to a chiral environment akin to the native state.

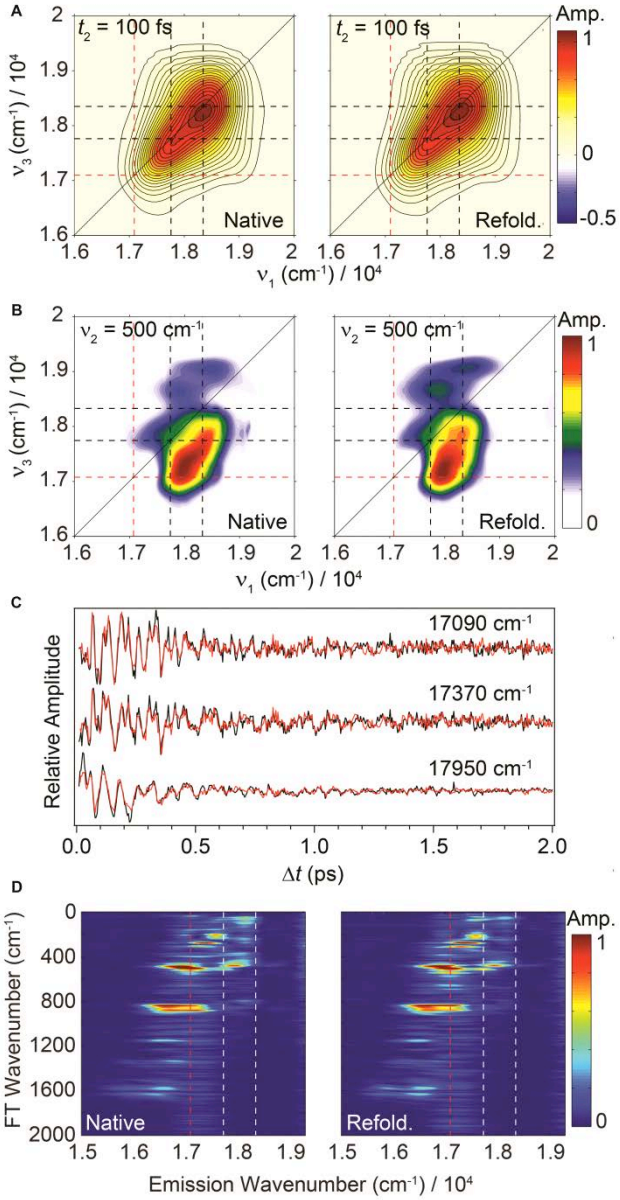

Figure 2. *Coherent spectroscopy of the light-harvesting PE545 protein. A) Normalized 2D spectra at t_2 = 100 fs. B) 2D coherence maps at 500 cm^{-1} for native (left) and refolded (right) proteins. C) Broadband transient absorption (BBTA) traces of native (black) and refolded (red) proteins at various spectral positions, where each trace is an average of five repeats. D) The power spectra for native (left) and refolded (right) proteins generated from coherent BBTA. Dashed lines indicate steady-state absorption (white) and fluorescence (red) maxima.*

Formation of hybrid cryptophyte light harvesting proteins

By recombining subunits separated by HPLC and refolding, we have generated hybrid proteins that we would expect to be either open (Figure 3) or closed (Figure 4). The spectra of the hybrid constructs indicate that the protein has properly refolded. Size exclusion chromatography analysis indicates that the hybrid protein differs from the native protein, it elutes at a higher volume indicating that it may be an αβ monomer rather than an $(\alpha\beta)_2$ dimer. We have obtained crystals of these hybrid proteins and we need to determine their three-dimensional structures in order to understand their make up. The proteins are soluble and stable and should be suitable for 2DES analysis as per the refolded wild type proteins.

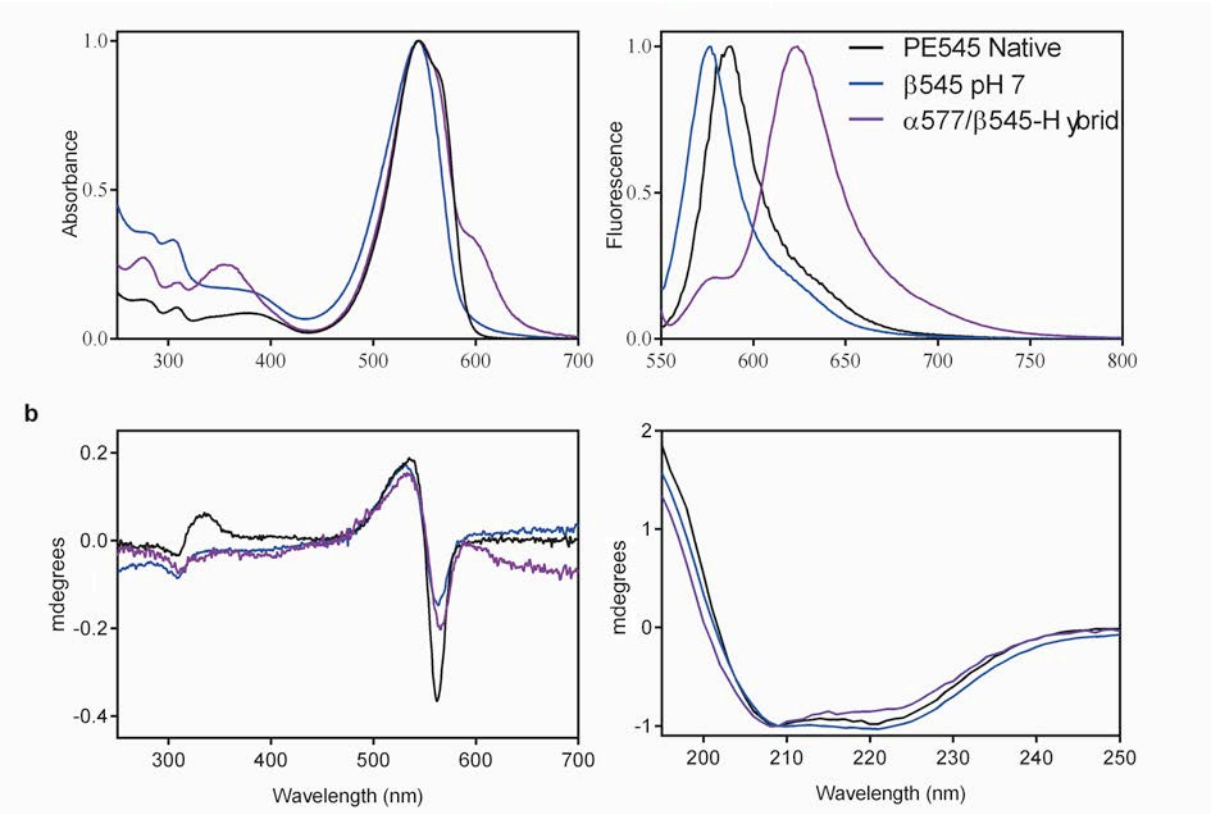

Figure 3. *Characterisation data of β_{545}/α_{577} open form hybrid (purple line), β_{545} (blue line) and PE545 (black line). (a) UV-Vis absorbance (left) and Fluorescence (right) (b) Circular Dichroism of chromophore region (left) and folding region (right).*

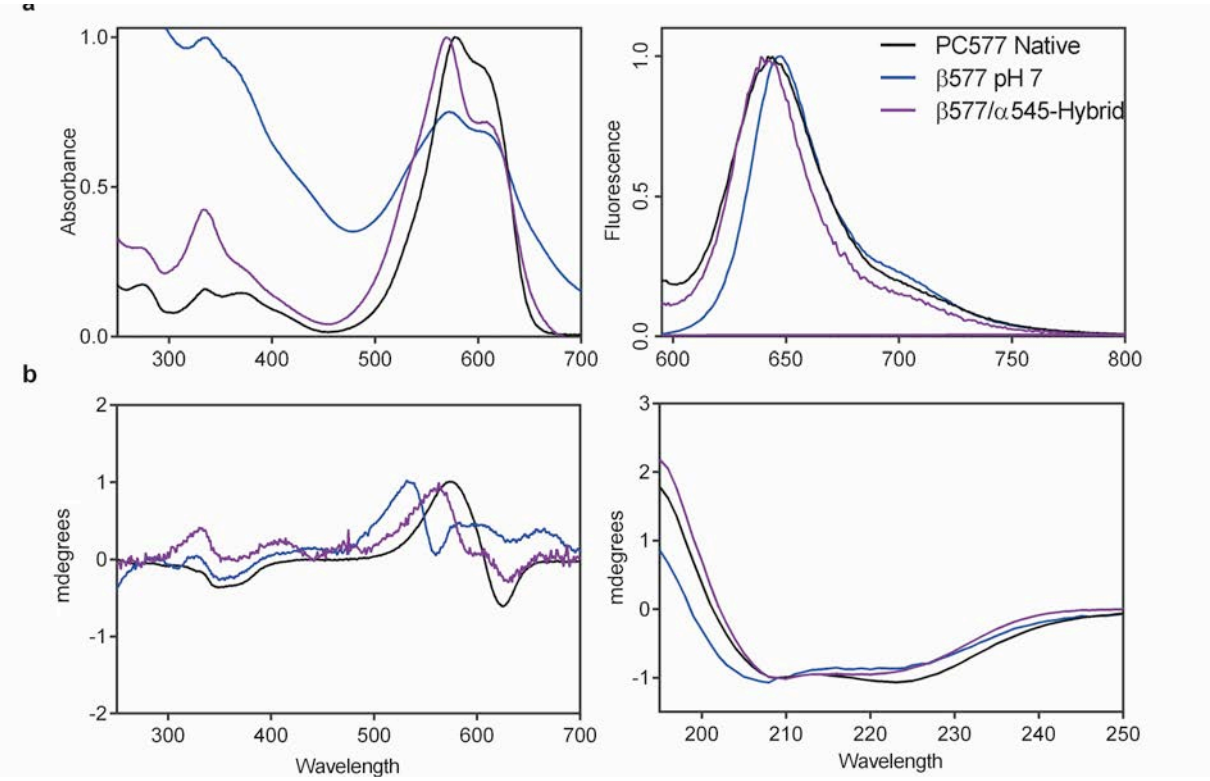

Figure 4. *Characterisation data of β_{577}/α_{545} closed form hybrid (purple line), β_{577} (blue line) and PC577(black line). (a) UV-Vis absorbance (left) and Fluorescence (right) (b) Circular Dichroism of chromophore region (left) and folding region (right).*

References

1. Lambert, N. et al. Quantum biology. *Nature Physics* **9**, 10-18 (2013).
2. Collini, E. et al. Coherently wired light-harvesting in photosynthetic marine algae at ambient temperature. *Nature* **463**, 644-7 (2010).
3. Brif, C., Chakrabarti, R. & Rabitz, H. Control of quantum phenomena: past, present and future. *New Journal of Physics* **12**(2010).
4. Ball, P. The dawn of quantum biology. *Nature* **474**, 272-274 (2011).
5. Curtis, B.A. et al. Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. *Nature* **492**, 59-65 (2012).
6. Apt, K.E., Collier, J.L. & Grossman, A.R. Evolution of the phycobiliproteins. *J Mol Biol* **248**, 79-96 (1995).
7. Huber, R. Nobel lecture. A structural basis of light energy and electron transfer in biology. *Biosci Rep* **9**, 635-73 (1989).
8. Glazer, A.N. Light guides. Directional energy transfer in a photosynthetic antenna. *J Biol Chem* **264**, 1-4 (1989).
9. Vesk, M., Dwarte, D., Fowler, S. & Hiller, R.G. Freeze-Fracture Immunocytochemistry of Light-Harvesting Pigment Complexes in a Cryptophyte. *Protoplasma* **170**, 166-176 (1992).
10. Ludwig, M. & Gibbs, S.P. Localization of phycoerythrin at the lumenal surface of the

thylakoid membrane in Rhodomonas lens. *J Cell Biol* **108**, 875-84 (1989).
11. Doust, A.B. et al. Developing a structure-function model for the cryptophyte phycoerythrin 545 using ultrahigh resolution crystallography and ultrafast laser spectroscopy. *J Mol Biol* **344**, 135-53 (2004).
12. Wilk, K.E. et al. Evolution of a light-harvesting protein by addition of new subunits and rearrangement of conserved elements: crystal structure of a cryptophyte phycoerythrin at 1.63-A resolution. *Proc Natl Acad Sci U S A* **96**, 8901-6 (1999).
13. Harrop, S.J. et al. Single-residue insertion switches the quaternary structure and exciton states of cryptophyte light-harvesting proteins. *Proc Natl Acad Sci U S A* **111**, E2666-75 (2014).

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